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14. ABSTRACT

Seventy seven 10 week old TRAMP mice were enrolled in the study. Administration of metronomic chemotherapy with cyclophosphamide and AN2 crossreacting mAb 225.28 or isotype control mAb F3-C25 was well tolerated. The therapy reduced disease burden and the severity of the disease. The findings from these studies support development of metronomic chemotherapy for the treatment of progressive disease (men with a rising PSA), aggressive forms of the disease and castration recurrent disease.

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Combinatorial targeting of prostate cancer cells and tumor associated pericytes with antibody-based immunotherapy and metronomic chemotherapy.

FINAL REPORT

INTRODUCTION

The lack of efficacy of conventional therapies in prostate cancer has stimulated interest in developing and implementing novel therapeutic strategies. Among them is immunotherapy. We have selected tumor antigen-specific monoclonal antibody (mAb)-based immunotherapy since a growing body of clinical evidence has convincingly shown that this type of immunotherapy is therapeutically effective for the treatment of some hematological diseases and solid tumors. One major limitation of immunotherapy is the negative impact of escape mechanisms on its outcome. To counteract this limitation of immunotherapy, the present proposal tested the hypothesis that the efficacy of immunotherapy of prostate cancer can be enhanced by targeting not only cancer cells, but also activated pericytes in the tumor microenvironment with a combinatorial immunotherapy. The latter includes continuous administration of low dose cyclophosphamide, i.e. metronomic chemotherapy, and administration of tumor antigen AN2-specific mAb. The tumor antigen selected as a target of immunotherapy is the mouse equivalent of the human chondroitic sulphate proteoglycan 4 (CSPG4). This antigen has a restricted distribution in normal tissues, but has high expression in malignant lesions with limited inter-and intra-lesional heterogeneity in various types of malignant diseases. The latter include glioma, head and neck squamous cell carcinoma, breast carcinoma, mesothelioma, melanoma and sarcoma. Relevant to this proposal AN-2 and CSPG4 are expressed in mouse and human prostate cancer cell lines, respectively (Fig. 1). Furthermore AN-2 and CSPG4 expression is upregulated on activated pericytes in the tumor microenvironment (Fig.2). As to the function of CSPG4 in cell biology, this antigen is involved in signaling pathways associated with cell survival, proliferation and migration. As a result, CSPG4-specific mAb inhibits tumor cell proliferation and migration *in vitro* and metastatic spread *in vivo*.

BODY

Model used to test the validity of the proposed strategy. Seventy-seven 10 week old TRAMP mice have been used. They were assigned to 3 cohorts: I.) 27 mice were treated with AN2 crossreacting mAb 225.28 and cyclophosphamide (CTX) (225.28+CTX); II.) 26 mice were treated with isotype matched control mAb F3-C25 and CTX (F3-C25+CTX); and III.) 24 mice were treated with vehicle alone (CONTROL). Cyclophosphamide (CTX) was administered at the dose of 10mg/kg/day in drinking water. AN-2 crossreacting mAb 225.28 or isotype control mAb F3-C25 (100 μg/injection) was administered i.p. every third day for 8 weeks.

Lack of toxicity of combinatorial therapy in TRAMP mice. After 8 weeks of treatment mice were weighed and humanely euthanized. The mean values ± SE of the weight of mice in the three groups is shown in Fig. 3. Non-parametric statistical analysis of the results detected no difference in the body weight among the 3 groups of mice, indicating the therapy was well tolerated in the animals.

Reduction by cyclophosphamide of prostate weight. After 8 weeks of treatment mice were humanely euthanized and reproductive tracts removed. The prostatic complex was microdissected from the reproductive tract under a stereomicroscope and the individual lobes of the prostate weighed. The weight of the dorsal (DP), lateral (LP), and ventral (VP) lobes were combined to give the prostate weight. The mean value ± SE of the prostate weight in the three groups of mice is shown in Fig. 4. Non-parametric statistical analysis of the results showed that the prostate weight in the groups of mice treated with mAb 225.28+ CTX or with mAb F3-C25+CTX was significantly ($P<0.002$) less than that in the control group. No difference was found in the prostate weight between the group of mice treated with mAb 225.28+CTX and that treated with isotype control mAb+CTX (mAb F3-C25+CTX).

Reduction in the disease score of prostate in mice treated with mAb 225.28+CTX or mAb F3-C25+CTX. Prostate tissues were formalin fixed and embedded in paraffin and processed for histological evaluation by hematoxylin and eosin staining. Histology was evaluated and each lobe of the prostate was given a tumor grade. Grade 1 represents normal prostate with secretory epithelial cells and an open lumen. Grade 2 has an open lumen but areas for transformed cells with a shift in the cytoplasmic to nuclear ratio and a piling up of the cells. In grade 3 all the epithelial cells have a transformed phenotype and the lumen is being filled with cells and a profound piling of the cells. Grade 4 represents carcinoma in situ with cells invading through the basement membrane. Grade 5 represent solid tumors with a glandular architecture and grade 6 are poorly differentiated tumors with sheets of anaplastic cells. The histological grade of the ventral prostate was evaluated. Each animal received a numerical tumor grade for worst, best and overall histological score. The three histological scores were averaged to give a disease score. The disease scores were compared among the treatment groups. Groups receiving mAb 225.28+CTX or isotype control mAb+CTX (mAb F3-C25+CTX) had a lower average disease score compared to control animals (Fig. 5).

Representative examples of lesions with low grade (LG), intermediate grate (IG) and high grade (HG) disease in mice treated with mAb 225.28+CTX or mAb F3-C25+CTX or the control group. LG disease lesions are characterized by piling up of cells in lumen, shift in the nuclear to cytoplasimic ratio and thin layer of tightly associated smooth muscle. IG disease lesions are characterized by filling of lumen with cells and irregular shape and hyperchromatism of nuclei. HG disease lesions are characterized by sheets of anaplastic cells. The ventral prostate was examined more in depth because it has been shown to be the most responsive to therapeutic intervention and demonstrated the most response in this study. The distribution of tumor grade was examined by low, intermediate and cancer. Low represents grades 1 and 2. Intermediate represents grade 3. Cancer represents grades 4, 5 and 6. Representative examples are shown in Fig. 6. The data for distribution is presented in Fig. 7. The incidence of cancer decreased and the frequency of low grade disease increased in animals treated with mAb 225.28+CTX or treated with isotype control mAb+CTX (mAb F3-C25+CTX) compared to control animals (Fig.7). It is noteworthy that there appeared to be less blood vessels in the cohort of mice treated with mAb 225.28+CTX as compared to the mice treated with the isotype mAb+CTX (mAb F3-C25+CTX) (Fig. 6).

KEY RESEARCH ACCOMPLISHMENT

- Metronomic chemotherapy with CTX + AN2 crossreacting mAb 225.28 or CTX + isotype control mAb F3-C25 are well tolerated.
- Metronomic chemotherapy with CTX + AN2 crossreacting mAb 225.28 or CTX + isotype control mAb F3-C25 reduces disease burden.
- Metronomic chemotherapy with CTX + AN2 crossreacting mAb 225.28 or CTX + isotype control mAb F3-C25 reduces the severity of the disease.

- Metronomic chemotherapy with CTX + AN2 crossreacting mAb 225.28 reduces the vasculature in the tumors.

REPORTABLE OUTCOMES

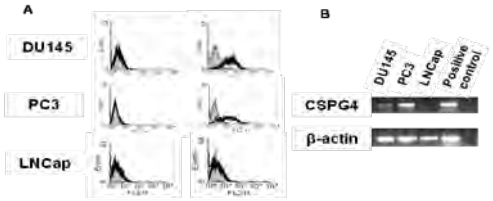
Administration of metronomic chemotherapy with CTX + AN2 crossreacting mAb 225.28 or CTX + isotype mAb F3-C25 delays prostate cancer progression reducing disease burden and the severity of the disease. Metronomic chemotherapy with CTX + AN2 crossreacting mAb 225.28 reduces the presence of mature vasculature in the tumors as evaluated by immunohistochemisty.

CONCLUSION

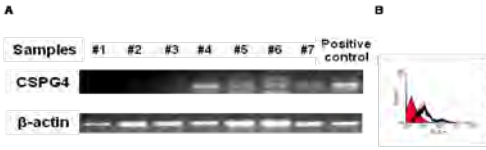
The results obtained thus far indicate that the therapeutic strategy outlined in the grant application is effective. The findings from these studies support development of metronomic chemotherapy for the treatment of human prostate cancer. Metronomic chemotherapy holds promise as a new therapeutic approach for the treatment of progressive disease (men with a rising PSA), aggressive forms of the disease and castration recurrent disease. Studies to elucidate the the mechanism of action underlying the therapeutic response are in progress.

Figure 1

CSPG4 and AN2 expression by human and mouse prostate cancer cells, respectively.



CSPG4 expression in human prostate cancer cells. *(A)* Empty histograms represent staining of prostate cancer cells with CSPG4-specific mAb 763.74. Grey histograms represent staining with isotype mAb control. *(B)* CSPG4 expression at the transcription level.

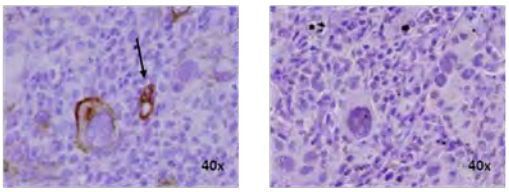


CSPG4 expression in TRAMP tumors. *(A)* CSPG4 expression is detected in TRAMP mouse model at the transcription level. (B) The empty histogram represents staining of mouse prostate cancer cells derived from TRAMP model with a cocktail of CSPG4-specific mAb. Red histograms represent staining with isotype mAb control.

Figure 2

CSPG4 upregulation on activated pericytes in the tumor microenvironment

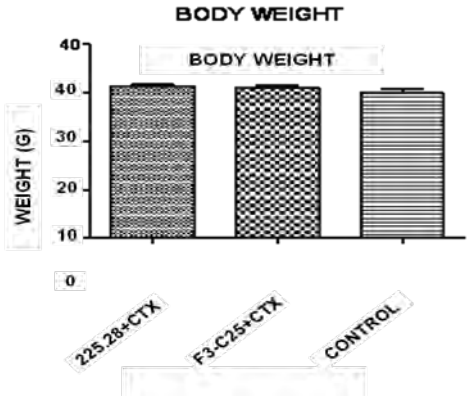
CSPG4 expression on pericytes and endothelial cells in a non-small cell lung carcinoma lesion



CSPG4-specific mAb Isotype control

Figure 3

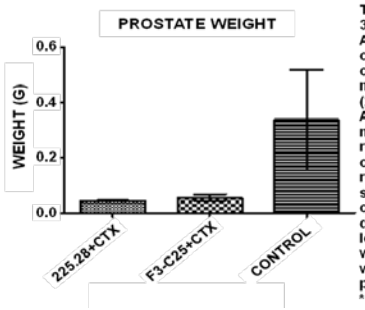
Lack of toxicity of combinatorial therapy in TRAMP mice



Ten week old TRAMP mice were assigned to 3 cohorts: one (27 mice) was treated with AN2 crossreacting mAb 225.28 and cyclophosphamide (CTX) (225.28+CTX), one (26 mice) with isotype matched control mAb F3-C25 and CTX (F3-C25+CTX) and one (24 mice) with vehicle alone (CONTROL). After 8 weeks of treatment mice were weighed and humanely euthanized. Data is reported as mean weight ± SE. Statistical analysis was performed using non-parametric statistics.

Figure 4

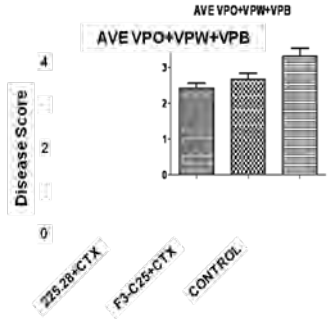
Effect of combinatorial therapy on prostate weight



Ten week old TRAMP mice were assigned to 3 cohorts: one (27 mice) was treated with AN2 crossreacting mAb 225.28 and cyclophosphamide (CTX) (225.28+CTX), one(26 mice) with isotype matched control mAb F3-C25 and CTX (F3-C25+CTX) and one (24 mice) with vehicle alone (CONTROL). After 8 weeks of treatment (18 weeks of age) mice were humanely euthanized and reproductive tracts removed. The prostatic complex was microdissected from the reproductive tract under a stereomicroscope and the individual lobes of the prostate weighed. The weight of the dorsal (DP), lateral (LP), and ventral (VP) lobes were combined to give the prostate weight. Data is reported as mean prostate weight ± SE. Statistical analysis was performed using non-parametric statistics. * P<.002

Figure 5

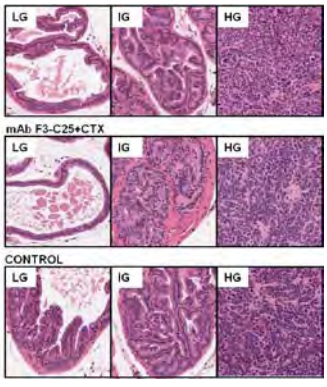
Disease Score with Treatment



Average disease score for animals treated with AN2 crossreacting mAb 225.28 and CTX (225.28+CTX), isotype matched mAb F3-C25 and CTX (F3-C25+CTX) or vehicle alone (CONTROL). Each animal received a numerical score (1-6) for worst, best and overall histological score. The average of the combined score was calculated. Data is presented as average score ± SEM.

Figure 6

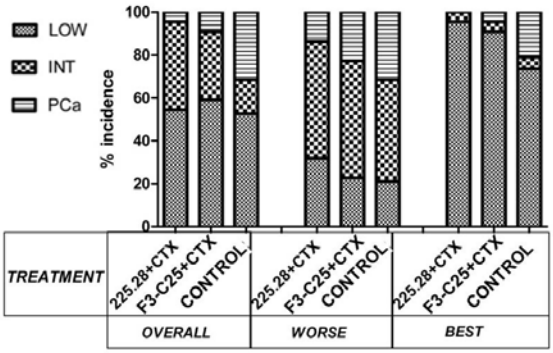
Representative examples of low, intermediate and high grade prostate lesions



Representative examples of lesions with low grade (LG), intermediate grade (IG) and high grade (HG) disease in mice treated with AN-2 crossreacting mAb 225.28 and CTX (225.28+CTX), isotype matched control mAb F3-C25 and CTX (F3-C25+CTX) or vehicle alone (CONTROL). LG disease lesions are characterized by piling up of cells in lumen, shift in the nuclear to cytoplasmic ratio and thin layer of tightly associated smooth muscle. IG disease lesions are characterized by filling of lumen with cells and irregular shape and hyperchromatism of nuclei. HG disease lesions are characterized sheets of anaplastic cells. Note: An interesting observation is that there appears to be less blood vessels in the cohort treated with 225.28+CTX compared to F3-C25+CTX or control.

Figure 7

Grade distribution with treatment



Distribution for ventral prostate grades in each cohort of mice treated with AN2 crossreacting mAb 225.28 and CTX (225.28+CTX), isotype matched mAb F3-C25 and CTX (F3-C25+CTX) or vehicle alone (CONTROL) . Each ventral prostate was given 3 disease scores representing the best histological score, the worst histological score and the overall score for that animal. The data is represented as distribution for each score. Note that with CTX treatment, the frequency of cancer decreases and the frequency of low grade disease increases.